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Teuvoa saxicola and *T. alpina* spp. nov. and the genus in China

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Abstract—Two new species, *Teuvoa saxicola* and *T. alpina*, are described from China. *Teuvoa saxicola* differs from other *Teuvoa* species by its saxicolous habitat, and its yellow brown, thick thallus. *Teuvoa alpina* resembles *T. junipericola* from western USA, but differs by its smaller ascospores and its higher altitude habitat. The morphological characters of the new species are illustrated. New material is described of *T. tibetica*, the only species previously recorded from China. The morphological and phylogenetic characteristics of the three species are discussed and compared with similar taxa.

Key words—*Megasporaceae*, *Pertusariales*, *Lobothallia*, lichens, taxonomy

Introduction

The lichen genus *Teuvoa* Sohrabi & S.D. Leav. (*Pertusariales*, *Megasporaceae*) was established based on the analysis of molecular sequence data and morphological characters (Sohrabi & al. 2013). The lichen family *Megasporaceae* has six genera: *Aspicilia*, *Circinaria*, *Lobothallia*, *Megaspora*, *Sagedia*, and *Teuvoa* (Schmitt & al. 2006, Lumbsch & al. 2007, Nordin & al. 2010, Sohrabi & al. 2013). Phylogenetic analysis by Sohrabi & al. (2013) supports *Teuvoa* as the sister group to *Lobothallia*.

Teuvoa is characterized by its 8-spored asci, absence of extrolites, rather short conidia and ascospores, lack of a subhypothecial algal layer, and different substratum preferences (Sohrabi & al. 2013). Worldwide, there are three described *Teuvoa* species: the terricolous *T. tibetica* and the epiphytic *T. junipericola* Sohrabi & S.D. Leavitt and *T. uxoris* (Werner) Sohrabi & al. (Rico & al. 2007; Sohrabi & al. 2010, 2013). During research on *Aspicilia* s. lat. in China's

arid and semi-arid regions, two saxicolous specimens from different localities and seven lignicolous specimens on conifers were collected. Morphological characters placed these specimens in *Teuvoa*, a placement confirmed by ITS sequence analyses. The saxicolous specimens are proposed here as a new species, *Teuvoa saxicola*, and the lignicolous specimens as a new species, *T. alpina*. New specimens are reported for *T. tibetica*, the only *Teuvoa* species previously recorded from China.

Material & methods

Morphological & chemical studies

Specimens collected from arid and semi-arid regions in northwestern China and preserved in the herbarium of Shandong Normal University, Jinan, China (SDNU), and the lichen herbarium of Herbarium Mycologicum, Chinese Academy of Sciences, Beijing, China (HMAS-L), were examined using an Olympus SZ51 dissecting microscope and Olympus CX41 compound light microscope. Apothecial sections were cut by hand and studied in water. Thalline cortex and medulla were spot tested (K, C, KC, I), and lichen substances were assessed in all samples by thin-layer chromatography (TLC) with solvent systems A, B, & C (Orange & al. 2001). Photographs were taken with a DP72 camera attached to an Olympus SZX16 stereomicroscope and an Olympus BX61 compound microscope.

Taxon sampling for DNA analyses

Nuclear ITS1-5.8S-ITS2 rDNA sequences of twenty-five specimens representing ten species were used in the molecular study. Six new sequences were produced from specimens collected in China and nineteen sequences were downloaded from GenBank (Table 1). *Lobothallia alphoplaca*, *L. crassimarginata*, *L. helanensis*, *L. melanaspis*, *L. radiosa*, and *L. recedens* were used as outgroup.

Extractions, PCR amplifications, sequencing

Total genomic DNA was extracted from the samples using the SanPrep Column DNA Gel Extraction Kit following the manufacturer's instructions. The complete ITS region (ITS1-5.8S-ITS2) was amplified using the primer ITS1F (Gardes & Bruns 1993) combined with ITS4 or ITS5 combined with ITS4 (White & al. 1990). The PCR thermal cycling parameters were initial denaturation for 3 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 52°C, 90 s at 72°C, and final elongation for 10 min at 72°C.

Amplification products were viewed on 0.8% agarose gels stained with ethidium bromide. The PCR amplicons were sequenced by Sangon Biotech Co. Ltd. with an ABI 3730 XL DNA Analyzer.

Sequence alignment & phylogenetic analyses

Sequences were assembled and edited using the program Geneious v6.1.2. Sequences were aligned using the program MAFFT v7 (Katoh & Toh 2008; Katoh & al. 2009). For ITS sequences, we used the L-ING-i alignment algorithm with the

Table 1. *Teuvoa* and *Lobothallia* species used in the phylogenetic analyses

Species	Locality	Voucher (Herbarium)	GenBank No.
T. alpina	China, Xinjiang	Li XL0306 (SDNU) [**T**]	**KX234706**
T. aff. *junipericola*	Iran	Sohrabi 9507B (hb. M. Sohrabi)	JX306740
T. junipericola	USA, Utah	Leavitt & Leavitt 850 (BRY–C54927)	JX306751
	USA, Utah	Leavitt & Felix 845 (BRY–C54926)	JX306750
	USA, Utah	St. Clair et al. 767 (BRY–C54923)	JX306747
	USA, Utah	St. Clair et al. 742 (BRY–C54922)	JX306744
	USA, Utah	Rosentreter 14521 (H) [**T**]	JX306741
T. saxicola	China, Qinghai	Ren 2722 (SDNU) [**T**]	**KX234703**
	China, Xinjiang	Ren 3352 (SDNU)	**KX234702**
T. tibetica	China, Xizang	Cheng 20116450 (SDNU)	**KX234705**
	China, Xizang	Cheng 20116469 (SDNU)	**KX234704**
	China, Qinghai	Ren 2730 (SDNU)	**KX234707**
	China, Xizang	Obermayer 04386 (H) [**T**]	GU289915
T. aff. *uxoris*	Turkey	Halici s.n. (hb. Halici)	JX306742
T. uxoris	Spain	Rico & Pizarro 3622B	JX306746
	Spain	Rico & Pizarro 3622 (H)	JX306743
	Spain	Rico & Pizarro 3622A	JX306745
L. alphoplaca	USA, Utah	Leavitt et al. 447 (BRY–C54921)	JX306737
	USA, Nevada	Leavitt & Leavitt 849 (BRY–C54920)	JX306739
L. crassimarginata	China, Nei Menggu	Wang 20122565 (SDNU)	JX476026
L. helanensis	China, Nei Menggu	Tong 20122791 (SDNU)	JX476031
L. melanaspis	Norway	Owe-Larsson 8943a	JF825524
	Sweden	Nordin 6622 (UPS)	HQ259272
L. radiosa	Sweden	Nordin 5889 (UPS)	JF703124
L. recedens	Sweden	Nordin 6035 (UPS)	HQ406807

Ex-type sequences are indicated by [**T**]. Newly obtained sequences are in **bold.**

remaining parameters set to default values. The alignments were analyzed by maximum likelihood (ML) and a Bayesian (BI) Markov chain Monte Carlo approach (B\MCMC). ML analyses were conducted in RAxML v8.2.6 (Stamatakis 2014). Bayesian analysis was performed using the software MrBayes v3.2.3 (Huelsenbeck & Ronquist 2001). Nucleotide substitution models were selected using the Akaike Information Criterion in jModelTest v2.1.7 (Darriba & al. 2012). Two B\MCMC analyses of two parallel runs were carried out for 10,000,000 generations. Each run included four chains, and trees were sampled every 1000 generations. All model parameters were unlinked. After discarding the burn-in, the remaining 7500 trees of each run were pooled to calculate a 50% majority-rule consensus tree. The clades that received bootstrap support (bs) ≥70% under ML and posterior probabilities (pp) ≥ 0.95 were considered well supported (Hillis & Bull 1993). Phylogenetic trees were visualized using FigTree v1.4.2.

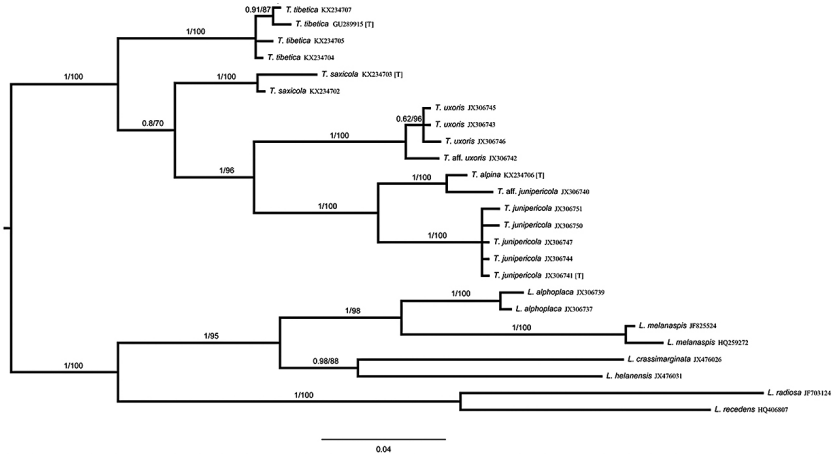

Fig. 1. Phylogenetic relationships within the genus *Teuvoa*, including the new species *T. saxicola* and *T. alpina*, with *Lobothallia* spp. outgroup. Phylogram inferred from Bayesian analysis of ITS dataset. Posterior probabilities ≥0.50 and ML bootstrap values ≥50% are indicated above internal branches. Ex-type sequences are annotated as [**T**].

Results & discussion

According to the results achieved from both analyses conducted with the ML with RAxML v8.2.6 and B\MCMC with MrBayes, we obtained congruent topologies; the BI topology is shown here (Fig. 1). In our phylogenetic trees, the *Teuvoa* representatives formed a strongly supported group sister to the *Lobothallia* representatives, further supporting segregation of *Teuvoa*. *Teuvoa* is divided into four well-supported clades, and the new species *T. saxicola* forms a highly supported clade (bs = 100%, pp = 1) within *Teuvoa*. The *Teuvoa junipericola* s.lat. lineage, strongly supported (bs = 96%, pp = 1) as sister to *T. uxoris,* is divided into two well supported clades, *T. junipericola* s.str. from western USA and *T. alpina* from northwestern China + *T.* aff. *junipericola* from Iran.

Taxonomy

Teuvoa saxicola Q. Ren, **sp. nov.** Fig. 2

Fungal Name FN570544

Differs from other *Teuvoa* species by its saxicolous habitat, and its yellow brown, thick thallus.

Type: China. Qinghai: Qilian Co., Yeniu Gou Town, Qilian mountain pass, 38°35.55′N 99°29.19′E, 4060 m, on siliceous rock, 21 Jun. 2013, Q. Ren 2722 (**Holotype**, SDNU; **isotype**, HMAS-L; GenBank KX234703).

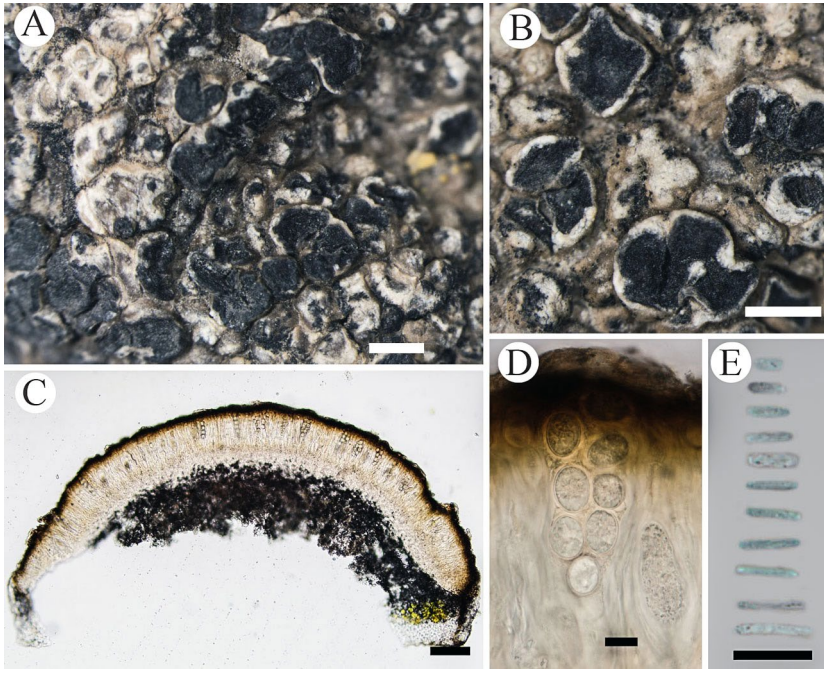

Fig. 2. *Teuvoa saxicola* (holotye, SDNU Ren 2722). A. Thallus with substratum; B. Apothecia; C. Section of apothecium; D. Ascus and ascospores; E. Conidia. Scale bars: A, B = 1 mm; C = 100 µm; D, E = 10 µm.

Etymology: The specific epithet refers to the substratum (siliceous rock) on which this new species grows.

Thallus saxicolous, crustose, continuous, rimose to areolate, sometimes verrucose, ≤3.5 mm thick. Areoles mostly irregular, sometimes angular to rounded, flat to convex, (0.25–)0.6–2 mm diam. Surface ochre to yellow brown, with a greenish tinge at the some parts of the thallus, slightly pruinose.

Apothecia numerous, one to several per areole, simple or composite, orbicular to slightly angular, 0.5–1.75 mm diam. Discs concave to plane, black, slightly pruinose. Thalline margin mostly prominent, elevated, sometimes indistinct, usually forming a black rim around the disc. Exciple ≤150 µm wide laterally, highly variable in development and thickness. Epihymenium dark brown, pigment N+ green, K+ brown. Hymenium hyaline, I+ persistently blue, 87.5–125 µm high. Subhymenium and hypothecium hyaline, I+ persistently blue, 25–37.5(–50) µm high. Paraphyses moniliform, with 3–7 globose to

subglobose cells apically. Asci clavate, *Aspicilia*-type, 8-spored. Ascospores hyaline, simple, globose or subglobose to ellipsoidal, 10–15 × 7.5–12.5 µm.

Pycnidia rare, immersed, rounded, with black ostioles. Conidia bacilliform, straight, 5–7.5(–10) × 0.7 µm.

Chemistry—Cortex and medulla K–, C–, KC–, I–. No secondary lichen substances detected by TLC.

Distribution & ecology—*Teuvoa saxicola* is known from Qinghai and Xinjiang provinces in northwestern China. It grows on siliceous rock in arid and semi-arid habitats.

Additional specimen examined—**CHINA. Xinjiang: Urumqi city,** Mt. Tianshan, Glacier No.1, 43°06.47′N 86°50.32′E, 3560 m, 8 Sept. 2013, Q. Ren 3352 (SDNU; Genbank KX234702).

Comments—The phylogenetic tree (Fig. 1) supports the two saxicolous specimens within a single well-supported lineage within *Teuvoa*. *Teuvoa saxicola* differs from other *Teuvoa* species by its saxicolous habitat, ochre or yellow brown thallus colour, and thick thallus. *Teuvoa tibetica* is a terricolous species with a white to whitish gray thallus and rhizomorph-like extensions. Both *T. uxoris* and *T. junipericola* are epiphytic species with a white to grey thallus (Sohrabi & al. 2013).

Teuvoa alpina Q. Ren, **sp. nov.** Fig.3

Fungal Name FN570545

Differs from *Teuvoa junipericola* s.str. by its smaller ascospores, and its higher altitude habitat.

Type: China. Xinjiang: Hami City, Baishitou, 2800 m, on coniferous wood, 28 Jul. 2013, Li XL0306 (**Holotype**, SDNU; **isotype**, HMAS-L; Genbank KX234706).

Etymology: The specific epithet refers to the high altitude at which this species grows.

Thallus epiphytic, crustose, continuous, rimose to areolate. Areoles mostly irregular, sometimes angular to rounded, flat to convex, 0.5–2.5 mm diam. Surface white to whitish grey with a greenish hue, smooth or slightly roughened, ± pruinose.

Apothecia numerous, cryptolecanorine or urceolate when young, becoming lecanoroid or lecideoid when mature, one to several per areole, simple or composite, orbicular to slightly angular, 0.5–2.0 mm diam. Discs concave to plane to slightly convex, brownish black to black, but appearing whitish to bluish grey due to pruina. Thalline margin mostly distinct, concolorous with thallus, thin, usually forming a white rim around the disc. Exciple highly variable in development and thickness, I+ blue. Epihymenium dark brown, pigment N+ green, K+ brown. Hymenium hyaline, I+ persistently blue, (75–)87.5–100 µm

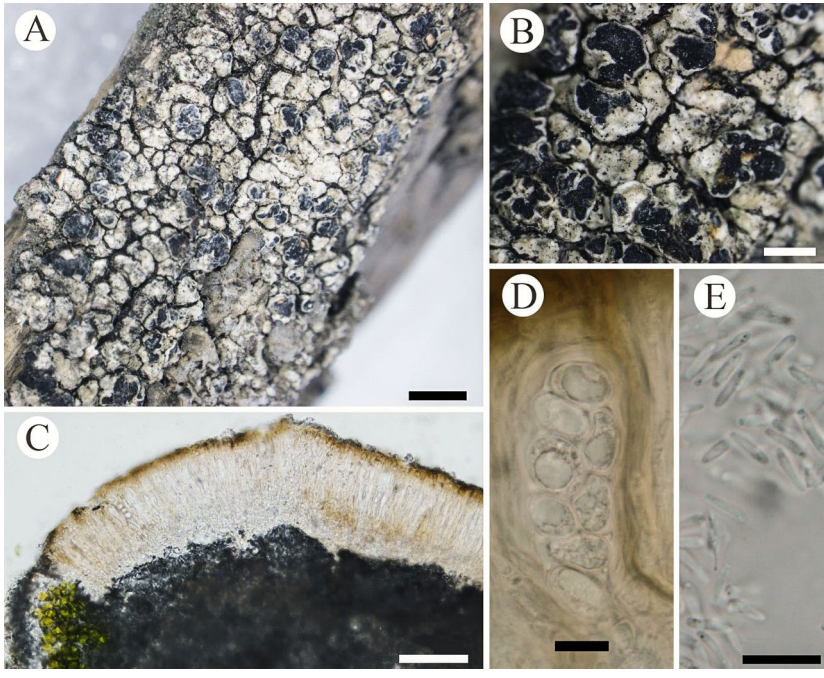

Fig. 3. *Teuvoa alpina* (holotye, SDNU Li XL0306). A. Thallus with substratum; B. Apothecia; C. Section of apothecium; D. Ascus and ascospores; E. Conidia. Scale bars: A = 2 mm; B = 1 mm; C = 100 μm; D, E = 10 μm.

high. Subhymenium and hypothecium pale, I+ persistently blue. Paraphyses submoniliform to moniliform, with 2–4 globose to subglobose cells apically. Asci clavate, *Aspicilia*-type, 8-spored. Ascospores hyaline, simple, ellipsoidal to subglobose, 10–15 × 7–10 μm. Pycnidia common, immersed, rounded, with black ostioles. Conidia bacilliform, straight, 5–7.5 × 0.7 μm.

Chemistry—Cortex and medulla K–, C–, KC–, I–. No secondary lichen substances detected by TLC.

Distribution & ecology—*Teuvoa alpina* is known from Xinjiang and Qinghai in northwestern China. It grows on coniferous wood in arid and semi-arid habitats.

Additional specimens examined—**CHINA. Xinjiang: Urumuqi City,** Mt. Tianshan, 2800 m, on coniferous wood, 29 July 1978, Wang 0103 (HMAS-L). **Qinghai. Qilian Co.,** Mt. Niuxinshan, 3050–3400 m, on coniferous wood, 11 Aug. 2007, Wang 20071462-1, 20071496-1, 20071026-1 (SDNU); Du 20071601-3 (SDNU); Shi 20071620-2 (SDNU).

Comments—Our phylogenetic tree (Fig. 1) includes *T. alpina* within a single well-supported lineage with *T.* aff. *junipericola* from Iran, which is sister to

T. junipericola from western USA (Sohrabi & al. 2013). As we did not examine the only specimen identified as *T.* aff. *junipericola* from Iran, we do not treat the Iranian material here. *Teuvoa alpina* closely resembles *T. junipericola* from western USA in general appearance, but *T. junipericola* has larger ascospores (13–22 × 10–16 µm) and occurs at lower altitudes (1700–2100 m; Sohrabi & al. 2013).

Teuvoa tibetica (Sohrabi & Owe-Larss.) Sohrabi, Lichenologist 45: 357 (2013).

Sohrabi & al. (2010) characterize *T. tibetica* by a crustose, white to pale gray thallus, a lower surface with pale rhizomorph-like extensions, short conidia (5–7 µm), small ascospores (10–14 × 6–9 µm), *Aspicilia*-type 8-spored asci, a brown epihymenium, a short hymenium (70–80 µm), non-moniliform to submoniliform paraphyses, and the lack of secondary substances.

Specimens examined—**CHINA. Xizang**: **Anduo Co.**, Mt. Tanggula, 4700–5300 m, on soil or mosses, 31 Jul. 2011, Cheng 20116223, 20116236, 20116446, 20116469, 20116481, 20116482 (SDNU): Jiang 20116364 (SDNU). **Qinghai**: **Qilian Co.**, Yeniu Gou Town, Qilian Mountain Pass, 38°35.55′N 99°29.19′E, 4060 m, on mosses, 21 Jun. 2013, Q. Ren 2730 (SDNU).

Comments—Our new material closely matched the holotype description (Sohrabi & al. 2010). *Teuvoa tibetica* was previously known only from Xizang (four localities) and Sichuan (one locality) in China; we have added records from a third province, Qinghai, and an additional locality in Xizang.

Teuvoa tibetica generally resembles *Megaspora verrucosa* (Ach.) Hafellner & V. Wirth, which differs by its usually concave discs and much larger ascospores (35–50 × 25–39 µm) with thicker spore walls (c. 1.5–2.5 µm; James & Fletcher 2009).

Acknowledgments

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